

## PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *Aeglemarmelos* EXTRACT AGAINST PATHOGENS

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### ABSTRACT

World is endowed with a rich wealth of medicinal plants. Man cannot survive on this earth for long life without the plant kingdom because the plant products and their active constituents played an important role. Herbs have always been the principal form of Medicine in India and presently they are becoming popular throughout the world. Plants continue to be a major source of commercially consumed drugs. The trend of using natural products has increased in recent years and the active plant extracts are frequently screened for new drug discoveries. *Aeglemarmelos*, corr.(FaM. Rutaceae) is commonly known as Bael in Bengal and Vilvam in tamil is distributed throughout India in dry forests, and also cultivated. *Aeglemarmelos*, corr.(FaM. Rutaceae) extract is used as a natural medicine. It is a cheaper and safe alternative source of drugs. Antibacterial activity of *Aeglemarmelos* leaves, fruits, bark and root extracts was determined using agar well diffusion method. It had significant antibacterial potency against the tested pathogens. In the present study the plant active components of *Aeglemarmelos* leaves, fruits, bark and root were extracted using Five different extraction solvents namely ethanol, ethyl acetate, chloroform, formaldehyde and distilled water. The phytochemical compounds such as reducing sugar, Tannins, Phlobatanins, terpenoidssaponins, alkaloids, flavonoids and poly phenols were revealed. Leaves, fruits, bark and root powder extract of *Aeglemarmelos* was studied by using five different solvents against the tested pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*. The formaldehyde extract of leaves fruits, bark and root (100%) was found to be most effective against *Bacillus subtilis* (28 mm), *Staphylococcus aureus* (36 mm), *Escherichia coli* (24mm), *Pseudomonas aeruginosa* (27mm), and *Candida albicans* (29 mm). All these preliminary reports warrant an in depth analysis of the usefulness of *Aeglemarmelos* as miracle drug against various ailments.

**KEYWORDS:** *Aeglemarmelos*, Phytochemical Screening, Anti Microbial Activity, Cotton Fabric & Pad Dry Cure Method

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### INTRODUCTION

Plants continue to be a major source of commercially consumed drugs. Even many synthetic drugs have their origin from natural plant products. The trend of using natural product has increased in recent years and the active plant extracts are screened for new drug discoveries (Ncube *et al.*, 2008). Several studies on different parts of *Aeglemarmelos* showed that the plant possesses anti-diarrhoeal, anti-diabetic, anti-inflammatory, antipyretic, analgesic, anticancer, radio protective and antimicrobial activities (Arul *et al.*, 2005). Limited information is available regarding antimicrobial activity of *Aeglemarmelos*. The antimicrobial agent of *Aeglemarmelos* extracts was reported to effectively kill or greatly reduce or eliminate the growth of *Vibrio cholera*, *Salmonella*

*typhimurium*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus fumigates*, *Aspergillusniger*etc. (Grierson *et al.*, 1999)

Currently, the antimicrobial activities of many plant species have been reported. Scientific evidence has brought about the possibilities of the utilization of plant extracts in the treatment of fungal and bacterial infections and the development of antibacterial and antifungal products. Screening technique of biologically active medicinal compounds have been conducted on well known species of plant used in traditional medicines and most plants have shown antibacterial activity. Recently most of the research conducted in traditional healers are very effective in spite of the fact that there is no scientific justification (Kelmansonet *al.*, 2000).

Therefore, present study is carried out to prepare eco friendly, natural antimicrobial product from *Aeglemarmelos* extract for antimicrobial textile application.

## MATERIALS AND METHODS

### Collection of Plant Materials (Rajasekaran *et al.*, 2008)

The *Aeglemarmelos* leaves, fruits, bark and root were collected from the natural habitat in Coimbatore. It was ensured that the plant was healthy and uninfected. The collected materials were washed under running tap water to eliminate dust and other foreign particles and to cleanse them, thoroughly and shade dried. The shade dried material was grounded into powder.

### Preparation of Plant Extract (Pandey and Mishra, 2011)

The powdered plant materials were dissolved in ethanol, ethyl acetate, chloroform, formaldehyde and water separately. About 1g of sample was dissolved in 10 ml of solvent separately. Mixtures were kept in the dark for three days at room temperature in sterilized beakers wrapped with aluminium foil to avoid evaporation and exposure to sunlight. After 3 days mixtures were filtered through What man no.1 filter paper and kept it in incubator for evaporation. All the dried extracts were dissolved in DMSO (Dimethyl sulphoxide) and stored it in dry place for further analysis.

### Culture Collection and Identification of Selected Pathogens

Pathogens like *Bacillus subtilis*sp., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* were obtained from Sri Ramakrishna Hospital, Coimbatore- India.

### Preliminary Screening of Medicinal Extracts for Antibacterial and Anti fungal Activity

#### Antimicrobial Susceptibility Testing (Jyothi and Rao, 2010)

The antimicrobial activity of the *Aeglemarmelos* Leaves, fruits, bark and root leaf extracts was determined using agar well diffusion method by following the known procedure. Muller Hinton agar was prepared and sterilized. Log phase culture of the test specimens of bacteria were swabbed over the agar surface using the sterile cotton swab. Small amount of diluted fungal suspension were poured over the media to spread uniformly on the surface.

Wells were made on the agar surface using sterile gel puncture and about 10µl for bacteria and 300µl for fungi of the *Aeglemarmelos* Leaves, fruits, bark and root extracts were loaded onto the wells. The plates were incubated at 37<sup>0</sup> C for 24 hours for bacteria and 28°C for 72 hours for fungal activity. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The antimicrobial activities of the different extracts were evaluated by comparing their zones of inhibition with standard antibiotic ampicillin and amphotericin B.

### **Phytochemical Analysis (Pandey and Mishra, 2011)**

Phytochemical analysis of extract for qualitative detection of alkaloids, flavonoids, steroid, volatile oil, glycoside, reducing sugar, tannins and saponins was performed by the extracts.

### **Application of Aeglemarmelos Extract for Antimicrobial Finishing on Cotton Fabrics**

#### **Pad Dry Cure Method (Thamaralet al., 2011)**

*Aeglemarmelos* extract was applied directly on destarched 40s count cotton fabric by pad dry cure method. The 40s count, desized 100% cotton fabric was cut to the size of 30x30 cm and immersed in the test solutions. *Aeglemarmelos* extract (2%) and Alum binder (6%) was used. The sample fabric was immersed in the solution for 5min and then it was passed through a padding mantle running at a speed of 15/min with a pressure of 1kgf/cm to remove excess solution. 100% wet pick up was maintained for all of the treatments. After padding the fabric was air dried and then cured for 3 mins at 80°C.

### **Physical Characterization of Finished Fabric**

The following procedures were studied to check the Physical characterization of the finished fabric

#### **Air Permeability of Finished Fabric (ASTM D37-2004)**

The extract treated and untreated fabrics were analyzed for their effectiveness in permeability of air at a relative humidity of 65% and temperature of 21°C.

#### **Tensile Strength-Grab Test of Finished Fabrics (ASTMD 5034-95-2001)**

A tensile testing machine was used and this test determined the breaking strength and elongation of most textile fabrics.

#### **Tear Strength of Finished Fabrics (ASTM D2261-96)**

The tearing strength of treated and untreated fabrics was measured by the tongue procedure using constant rate of extension tensile testing machine. The force to continue the tear was calculated from autographic chart recorded.

### **Wash Durability Test**

The durability of antimicrobial activity of the dyed samples was evaluated after different wash cycles. The samples were washed with 5% neutral soap solution for 20 minutes. Washed samples were tested for the retention of antimicrobial activity after 2,4,6,8 and 10 laundering by AATCC -100 test method. Change in colour of the specimen was evaluated by comparison with Gray scale.

### **Evaluation of Antimicrobial Activity of Finished Fabric**

Sterile AATCC Bacteriostasis agar was prepared, sterilized and dispensed in sterile Petri dishes. Log phase culture of the test organisms *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229) were used as inoculum. Using sterile 4mm inoculating loop, one loop full of culture was loaded and transferred to the surface of agar plate by making five parallel inoculum streaks approximately 60mm in length and spaced 10mm covering the central area of petridish without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streak to ensure intimate contact with agar surface. The plates were incubated at 37°C for 18-24 hours.

The incubated plates were examined for the interruption of growth over the inoculum. The size of the clear zone was used to evaluate the inhibitory effect of the test sample and the average width of the zone along the streak on either side of the test specimen can be calculated by the following formula,

$$W = (T-D)/2$$

where, W is the width of clear zone of inhibition in mm.

T is the total diameter of test specimen and clear zone in mm.

D is diameter of the test specimen in mm.

## RESULTS AND DISCUSSIONS

### Maintenance of Plant Extract

Dried and ground samples were dissolved in DMSO and stored at room temperature in dry condition. The plant extracts were screened for antimicrobial activity.

### Cultural Characteristics

Based on the cultural characteristics, colony morphology, growth features and microscopical analysis the organisms were identified as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*.

### Antimicrobial Susceptibility Testing

Among all the solvents and part of *Aegle marmelos*, the formaldehyde leaf extract (Table 1) showed excellent activity against all pathogens.

**Table 1: Antimicrobial Susceptibility Testing**

S. No	Organism	Zone of Inhibition in mm
1	<i>Bacillus subtilis</i> .	28mm
2	<i>Staphylococcus aureus</i>	36mm
3	<i>Escherichia coli</i>	24mm
4	<i>Pseudomonas sp.</i> ,	27mm
5	<i>Candida albicans</i>	29mm

### The Phytochemical Analysis of *Aegle marmelos* Extracts

The leaves, fruits, bark and root extracts were screened for some secondary metabolites. The table 2 shows the Phytochemical analysis of *Aegle marmelos* extracts.

### Evaluation of Antimicrobial Activity of Finished Fabric

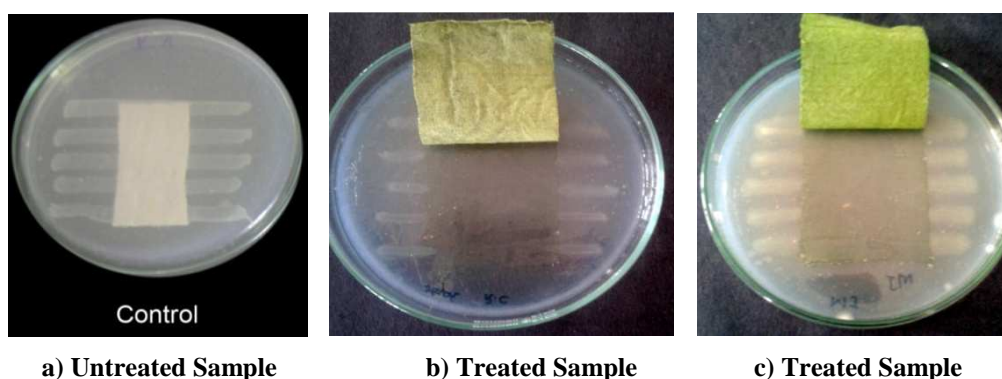
The formaldehyde leaf extract of *Aegle marmelos* was coated directly on to the cotton fabric and its efficacy was evaluated by Qualitative tests.

**Table 2: The Phytochemical Analysis of *Aeglemarmelos* Extracts**

S. No	Tests	Leaves	Fruit	Bark	Root
1	Reducing sugar	-	+	+	+
2	Tannins	+	+	-	-
3	Phlobatannins	+	+	+	-
4	Saponins	+	-	+	+
5	Terpenoids	+	+	+	-
6	Alkaloids	+	-	+	+
7	Flavanoids	-	-	+	+
8	Polyphenols	+	+	+	+

#### Qualitative Tests-Parallel Streak Method (AATCC Test Method 147-2007)

The cotton fabric finished with formaldehyde leaf extract of *Aeglemarmelos* (b and c) inhibited the growth of *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922). The result showed that there was no growth under the treated fabric. (Figure b and c)



**Figure 1: Physical Characterization of Cotton Fabric Finished with Formaldehyde Leaf Extracts of *Aeglemarmelos***

#### Air Permeability of Finished Fabric (ASTM D 737 - 2004)

Based upon the results (Table 3) air permeability of the cotton fabric was not much affected after the treatment using alum and formaldehyde leaf extract of *Aeglemarmelos*.

#### Tensile Strength-Grab Test of Finished Fabrics (ASTM D5034-95-2001)

Tensile strength value of control and finished fabrics in (Table 3). In treated fabric, the wrap strength reduction occurs from 41.95 Kg to 34.28 and warp elongation was found to be 1.56%.

#### Tear Strength of Finished Fabrics (ASTM D 2261-96)

The tear strength of cotton fabric finished with the formaldehyde leaf extract of *Aeglemarmelos* were given in Table (3). A less tear strength difference was noted in the treated samples both in wrap and weft ways.

**Table 3: Physical Characterization of Cotton Fabric Finished with Formaldehyde Leaf Extracts of *Aeglemarmelos***

S. No	Experiments	Control Fabric	Treated Fabric
1.	<b>Air permeability</b>	48.3	43.2
2.	<b>Tensile strength</b>		
	Wrap strength (kg)	41.95	34.28
	Wrap elongation (%)	13.17	11.61
	Weft strength(kg)	32.86	22.95
	Weft elongation (%)	19.95	17.58
3.	<b>Tear strength</b>		
	Wrap strength(gf)	1472.0	1290.0
	Weft strength(gf)	1392.0	1170.0

### Wash Durability Test

The fabrics treated with formaldehyde leaf extract of *Aeglemarmelous* sustained antibacterial activity against all the test bacteria until 22 washes. After that there occurred a slight reduction in the activity of the fabric.

### CONCLUSIONS

Approximately 80% of people in developing countries still rely on traditional medicines for their primary health care. This usually involves the use of plant ex-tracts. Medicinal plants usually constitute an important source of new and biologically active compounds. In the present study, the antimicrobial efficiency of different solvent extract of *Aeglesmarmelos* leaves, fruits, bark and root were studied. The results showed promising use of these herbal extracts as source of antimicrobial finishing on cotton fabric. The formaldehyde leaf extract finished fabric showed maximum antibacterial activity against all selected pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these herbal extracts in providing antimicrobial activity to the finished fabric.

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